Virus Genes (2007) 35:235–242
DOI 10.1007/s11262-006-0044-1

Sequence and phylogenetic analysis of the S1 genome segment of turkey-origin reoviruses

J. Michael Day · Mary J. Pantin-Jackwood · Erica Spackman

Received: 13 September 2006 / Accepted: 20 September 2006 / Published online: 30 January 2007

Abstract Based on previous reports characterizing the turkey-origin avian reovirus (TRV) σB (σ2) major outer capsid protein gene, the TRVs may represent a new group within the fusogenic orthoreoviruses. However, no sequence data from other TRV genes or genome segments has been reported. The σC protein encoded by the avian reovirus S1 genome segment is the cell attachment protein and a major antigenic determinant for avian reovirus. The chicken reovirus S1 genome segment is well characterized and is well conserved in viruses from that species. This report details the amplification, cloning and sequencing of the entire S1 genome segment from two and the entire coding sequences of the σC, *p10* and *p17* genes from an additional five TRVs. Sequence analysis reveals that of the three proteins encoded by the TRV S1 genome segment, σC shares at most 57% amino acid identity with σC from the chicken reovirus reference strain S1133, while the most similar p10 and p17 proteins share 72% and 61% identity, respectively, with the corresponding S1133 proteins. The most closely related mammalian reovirus, the fusogenic Nelson Bay reovirus, encodes a σC protein that shares from 25% to 28% amino acid identity with the TRV σC proteins. This report supports the earlier suggestion that the TRVs are a separate virus species within the *Orthoreovirus* genus, and may provide some insight into TRV host specificity and pathogenesis.

Keywords Orthoreovirus · Turkey reovirus · p10 · p17 · SigmaC

NC/SEP–R44/03 S1 genome segment DQ525419
NC/98 S1 genome segment DQ995806
TRV sigmaC sequences DQ996601–DQ996605
TRV p10 sequences DQ996606–DQ996610
TRV p17 sequences DQ996611–DQ996615

J. M. Day · M. J. Pantin-Jackwood · E. Spackman (✉)
Southeast Poultry Research Laboratory, USDA-ARS, 934 College Station Road, Athens, GA 30605, USA
e-mail: espackman@seprl.usda.gov

Introduction

Avian reoviruses (ARVs) cause economically important diseases in poultry and belong to the genus *Orthoreovirus* in the family *Reoviridae*. Avian reoviruses contain a segmented double stranded RNA (dsRNA) genome comprised of three large (L1–L3), three medium (M1–M3) and four small (S1–S4) genomic segments; each of the ten reovirus genome segments encodes a single gene, with the exception of the S1 segment, which usually has three overlapping open reading frames (ORFs) [1]. ARVs generally differ from mammalian reoviruses in their ability to cause cell-to-cell fusion (they are fusogenic).

In poultry ARVs can cause tenosynovitis, respiratory disease, immunosuppression and enteric disease and are therefore of significant economic importance to the poultry industry [2]. ARVs have also been isolated from chickens and turkeys in flocks exhibiting no clinical signs of disease. Because of their economic importance vaccination is widespread in chickens and most vaccines are made from the well-described S1133 strain or from antigenically related isolates. Recently, a group of turkey-origin avian reoviruses (TRVs) have been described that are genetically distinct from other reoviruses of the *Orthoreovirus* family based on sequence analysis of the σB (σ2) gene encoded by the TRV S3 genome segment

[3, 4]. These TRVs were originally isolated from commercial turkey flocks affected by poult enteritis complex (PEC), a disease syndrome with uncertain etiology that is of economic importance to the turkey industry, especially in the southeastern United States [5]. These TRVs can induce enteric disease and immunosuppression in turkeys, however they do not appear to cause disease in chickens, and some strains may not be able to replicate in chickens [6].

The present study was undertaken to molecularly characterize the S1 genome segments and the predicted σC, p10 and p17 protein sequences from several of these TRVs. The σC protein is a minor outer capsid protein in the non-enveloped ARV virion, and is the protein responsible for initial attachment to host cells. Although the carboxy-terminal cell-binding domain of avian reovirus σC has recently been elucidated structurally [7, 8], the avian host cell receptor for reovirus is still unknown. ARV p10 proteins are members of the fusion-associated small transmembrane (FAST) protein family [9], and while the function of ARV p17 proteins is questionable, recent research shows that p17 contains a nuclear localization signal (NLS) [10]. Alignments of the TRV S1 genome segment and σC nucleic acid and predicted amino acid sequences with chicken-origin reovirus and mammalian fusogenic reovirus sequences supports earlier suggestions that the TRVs constitute a separate virus species within the *Orthoreovirus* genus, and further show that the TRV σC protein has diverged from the chicken sequence to a greater extent than have the TRV σB (σ2), p10 and p17 proteins. Despite the extensive sequence divergence, the TRV S1 segment putative proteins have conserved residues and structural motifs that may be important for virion assembly, host specificity, and host cell attachment.

Materials and methods

Viruses

The identification and isolation of TRV strains NC/98, NC/PEMS/85, TX/98 and TX/99, has been described previously [3, 4]. The TRV strains NC/SEP-R44/03, NC/SEP-R61/03, and NC/SEP-R108/03 were recently isolated from commercial turkeys in the southeastern United States and were propagated in Vero cells as previously described [6].

Isolation of viral RNA from turkey-origin reovirus

Each strain of turkey-origin avian reovirus was propagated in Vero cells for no more than four passages. Upon the development of >80% cytopathic effect (CPE), the remaining adherent cells were scraped and all cellular material was pelleted and frozen at –80°C until total RNA was extracted. Total RNA was extracted from thawed virus-infected cells using TRIzol® Reagent (Invitrogen Inc., Carlsbad, CA) according to the manufacturer's recommendations. RNA was reconstituted in nuclease-free water (Ambion) and ssRNA was precipitated with 2M LiCl (final concentration) overnight at 4°C. The ssRNA was pelleted via centrifugation and the viral dsRNA in the resulting supernatant was precipitated with 4M LiCl (final concentration) at –20°C for one hour. The precipitated viral dsRNA was pelleted via centrifugation, washed with 70% ethanol, dried and reconstituted in nuclease-free water and stored at –20°C. The concentration of the genomic viral RNA was determined spectrophotimetrically and the integrity of the RNA was checked via agarose gel electrophoresis.

Preparation of cDNA from isolated S1 genome segment

Purified turkey-origin reovirus genomic dsRNA was separated via electrophoresis on a 1.5% agarose gel. The S1 genome segment was excised and the dsRNA was isolated (Qiagen Gel Extraction Kit). The concentration of the dsRNA was determined spectrophotimetrically. The cDNA synthesis was facilitated via the ligation of an adaptor primer (5′-PO_4 AGG TCT CGT AGA CCG TGC ACC AmC7-3′) to each end of the dsRNA to serve as a template during reverse transcription. The adaptor primer was blocked by an amino modification at its 3′ end. A primer complementary to the adaptor (5′ GGT GCA CGG TCT ACG AGA CCT 3′) was used in subsequent RT-PCR steps. Ligation reactions contained 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 1 μM adaptor primer, 1 ng S1 genome segment, and 10U T4 RNA ligase (New England Biolabs). Reactions were incubated at 16°C overnight. Ligation reaction components were removed by RNA purification with the RNeasy Kit (Qiagen) and the ligated S1 genome segment was reconstituted in 10 μl nuclease-free water. Next, 8.5 μl of the ligated S1 genome segment was mixed with 1.5 μl 100% DMSO, heated to 99°C for 1 minute, and immediately placed on ice. RT-PCR was performed using the Qiagen One-Step RT-PCR kit. Reactions contained 10 μl 5× Qiagen RT-PCR buffer, 2 μl dNTP mix (10 μM each dNTP), 6 μl complementary adaptor primer (10 μM), 2 μl enzyme mix, 2.5 μl $MgCl_2$ (25 mM), 10 μl heated and cooled RNA/DMSO mixture, and 17.5 μl water. RT-PCR was performed at 55°C for 60 min, followed by

95°C for 15 min, and 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min with a final extension at 72°C for 10 min. The resulting cDNA of the proper size was cloned into the vector pCR2.1 TOPO (Invitrogen) and transformed into chemically competent *E. coli* DH5α.

The cDNA corresponding to both strands of the NC/98 and NC/SEP-R44/03 S1 genome segments was sequenced using fluorescently labeled dideoxynucleotides, *Taq* polymerase, oligonucleotide primers necessary to complete the entire segment, and an AB-3730 automated sequencer. The consensus nucleotide sequence of the NC/98 S1 genome segment was used to design TRVσC, *p10* and *p17* gene specific RT-PCR primers. These primers were used to amplify and directly sequence σC, p10 and *p17* cDNA from five additional TRV isolates: TX/98, TX/99, NC/SEP-R108/03, NC/SEP-R61/03 and NC/PEMS/85.

Sequence analysis and ORF prediction

Nucleotide and predicted amino acid sequence manipulations of S1 genome segments and putative σC, *p10* and *p17* genes were performed using Lasergene 99 v. 6.1 (DNASTAR). Nucleotide and amino acid identities were determined in MegAlign after a multiple alignment using ClustalW [30]. Multiple alignment figures of putative protein sequences were performed with ClustalX [31] and shaded using BOXSHADE. Phylogenetic trees were created in PAUP* (4.0b10) [32] using maximum parsimony and 2000 bootstrap replicates. The GenBank accession numbers for the reovirus sequences included in these comparisons are as follows: duck (AJ310525), goose (AJ717735), NBV (AF218360), S1133 (AF330703), ARV 176 (AF218358), ARV 1733 (AF004857), GEL13A98M (AF354226).

Results

Phylogenetic analysis

Phylogenetic analysis of the putative TRV σC proteins demonstrates that the TRV isolates NC/SEP-R108/03, NC/SEP-R61/03, NC/98, NC/PEMS/85, TX/99 and TX/98 form a separate group that is distinct from previously reported chicken, goose, duck, and fusogenic mammalian isolates (Fig. 5). This is in agreement with an earlier analysis performed, using the sequence of the σB (σ2) protein gene from selected TRV isolates [4]. These TRV isolates also group closely with a German avian reovirus isolate, Gel13a98m, which was isolated from chickens experiencing malabsorption syndrome (MAS) [11]. Interestingly, of the isolates chosen for the present comparison, the NC/SEP-R44/03 σC groups closely with σC from Nelson Bay Reovirus (NBV), a flying fox isolate.

S1 genome segment nucleotide analysis

The nucleotide sequence of cDNA generated from the entire S1 genome segment of TRV isolates NC/98 and NC/SEP-R44/03 was determined. There was 32.8% nt identity between NC/98 and NC/SEP-R44/03 over the entire S1 segment. The NC/98 and NC/SEP-R44/03 S1 genome segments both contained a 5′ terminal conserved nucleotide sequence characteristic of the orthoreoviruses: 5′GCTTTTT for both NC/98 and NC/SEP-R44/03 (Fig. 1). TRV strain NC/SEP-R44/03 lacks the 3′ terminal C residue in the 3′ consensus sequence; NC/98 contains the full consensus 3′ sequence (TATTCAT3') (Fig. 1). Over the entire approximately 1.6 kb S1 genome segment, S1 from isolates NC/98 and NC/SEP-R44/03 shared 54.7% and 33.7% nucleotide identity, respectively, with S1 from the reference (chicken-origin) ARV strain S1133. Nucleotide identities were essentially the same, 54.7% and 33.5% for NC/98 and NC/SEP-R44/03, respectively, when the two isolates were compared to the avian reovirus 1733 S1 sequence. Avian reovirus 1733 is a very pathogenic avian reovirus strain that produces high morbidity and mortality in chickens [12]. Of the S1 genome segments included in this comparison, only the S1 segment from Nelson Bay reovirus, a fusogenic mammalian reovirus, had a lower nucleotide identity than the TRVs when compared to the S1133 and 1733 sequences (30.4% and 30.1%, respectively). Interestingly, the NC/98 and NC/SEP-R44/03 S1 sequences were quite different when compared to one another, with only a 32.8% identity at the nucleotide level.

Based upon predicted open reading frames (ORF) start and stop codons, the S1 genome segments from NC/98 and NC/SEP-R44/03 each contain the three ORF's reported for numerous other orthoreovirus S1 segments. These ORFs have a partially overlapping gene arrangement and consist of the *p10*, *p17*, and σC ORFs arranged from 5' to 3' on the S1 genome segment plus strand (mRNA) [1].

```
S1133   5′-GCTTTTT…TATTCATC-3′
1733    5′-GCTTTTT…TATTCATC-3′
NBV     5′-GCTTAAT…TACTCATC-3′
NC98    5′-GCTTTTT…TATTCATC-3′
SEP44   5′-GCTTTTT…TATTCAT•-3′
```

Fig. 1 Comparison of the conserved 5′ and 3′ terminal sequences of the S1 genome segments from several fusogenic orthoreoviruses, including the turkey-origin reoviruses NC/98 and NC/SEP-R44/03

Sequence analysis of the p10, p17 and σC predicted amino acid sequence

The amino acid identity of the predicted TRV σC proteins when compared to the S1133 σC was 55.4% and 35.5% for NC/98 and NC/SEP-R44/03, respectively. The σC amino acid identity among the five additional TRV isolates and the S1133 σC was similar to that observed for NC/98 (approximately 53 to 56%). Interestingly, the σC amino acid identities among the TRV isolates included in this study ranged from approximately 75% up to 98%, with the exception of the NC/SEP-R44/03 isolate, which shared from 35% to 37% amino acid identity with the other TRV isolates (Fig. 2). This is comparable to the identity NC/SEP-R44/03 shared with S1133 σC (35.8%). Despite the divergence noted among these newly sequenced TRV σC proteins, a number of predicted amino acids were conserved in all isolates, and residues that were not conserved in all cases were usually substituted with a similar amino acid (Fig. 3). There was a great deal of conservation in residues found in the heptad repeat motif in the N-terminal portion of the σC monomer, particularly at positions "a" and "d" of the heptad. The C-terminal portions of the TRV σC monomers contain a number of universally conserved aromatic amino acid residues (Fig. 3).

% sigmaC amino acid identity

	1. S1133	2. SEP61	3. TX 98	4. NC98	5. NC85	6. SEP108	7. TX99	8. SEP44	9.GEL13A	10. NBV
1. S1133	100.0									
2. SEP61	56.6	100.0								
3. TX 98	53.2	85.3	100.0							
4. NC98	55.4	95.4	85.6	100.0						
5. NC85	53.8	85.9	98.8	86.2	100.0					
6. SEP108	55.0	95.4	85.9	96.9	86.5	100.0				
7. TX99	55.7	88.1	90.8	88.4	91.4	89.0	100.0			
8. SEP44	35.5	36.1	35.5	35.5	35.5	36.7	35.5	100.0		
9. GEL13A	53.2	77.1	78.6	76.8	78.6	77.7	79.2	35.5	100.0	
10. NBV	24.8	26.0	26.3	25.7	26.3	26.0	26.0	27.6	26.6	100.0

Fig. 2 Amino acid identity comparison of the predicted σC amino acid sequences from several orthoreoviruses, including seven turkey-origin reoviruses, Nelson Bay Reovirus, and a German-origin avian reovirus (GEL13A)

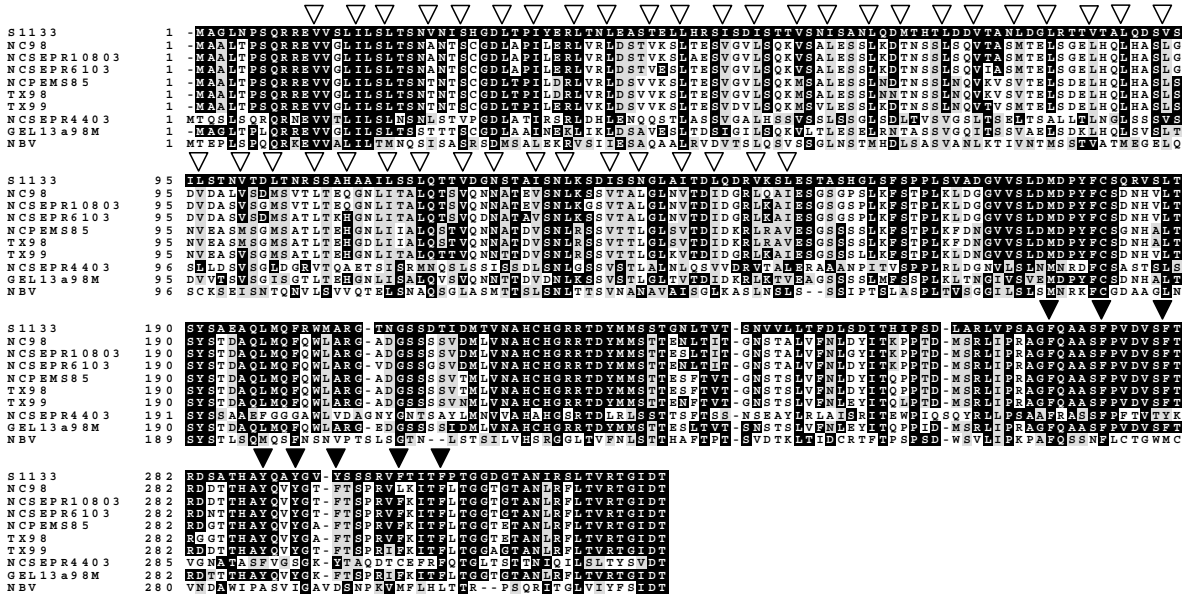

Fig. 3 Multiple sequence alignment of reovirus σC proteins, including the predicted σC amino acid sequences for seven turkey-origin reoviruses: NC/SEP-R61/03, NC/SEP-R108/03, NC/PEMS/85, NC/98, TX/98, TX/99 and NC/SEP-R44/03. GEL13a98M is a reovirus of German origin (11) and NBV is Nelson Bay Reovirus, a fusogenic mammalian reovirus. The residues that form the conserved heptad repeat in the σC monomer are indicated by an open triangle. Conserved aromatic amino acids located in the C-terminal portion of the σC monomer are indicated with closed triangles. Residues identical to the S1133 σC amino acid sequence are shaded in black, and gray shading indicates conservative amino acid substitutions

The predicted p10 amino acid sequences for the NC/SEP-R44/03 and NC/PEMS/85 isolates shared only 54.5% and 62.0% identity, respectively, with the p10 protein from S1133, while the p10 amino acid sequences from the remaining isolates shared from 68.7 to 71.7% identity with the S1133 p10. The TRV p10 proteins all contain discernable putative transmembrane domains, although the domain is not 100% conserved compared to the domain present in the S1133 and 1733 p10 amino acid sequences (Fig. 4A). The predicted p17 amino acid sequence for NC/SEP-R44/03 shared 35.6% identity with the S1133 p17 protein, while the remaining isolates shared from 57.3 to 59.6% identity. Although the p17 gene has been found in the S1 genome segment of all avian reovirus sequences to date, it has no homology to any described protein and its specific role during infection remains unclear. However, the avian reovirus p17 protein does contain a nuclear localization signal (NLS) and it has been recently revealed that p17 shuttles between the nucleus and the cytoplasm during an infection [10]. The TRVs all appear to contain this conserved NLS (Fig. 4B).

Discussion

The fusogenic orthoreoviruses are a very diverse group of dsRNA viruses that include the avian reoviruses, fusogenic mammalian reoviruses like Nelson Bay reovirus and baboon reovirus, and the reptilian reoviruses [13–15]. The reovirus cell attachment protein (σC in the avian orthoreoviruses, σ1 in the mammalian reoviruses) is the most variable of all the reovirus proteins and antigenic variation in this protein is the major factor in the inability of avian reovirus vaccine strains to confer full protection to commercial flocks [16–18]. The group of TRVs included in the present study exhibit a high degree of variability in their σC proteins, with amino acid identities among the isolates ranging from approximately 35% to 98%. The amino acid identity between the TRV NC/SEP-R44/03 and the other TRVs is only about 36% in all cases, which is comparable to its identity with the chicken reovirus reference strain S1133 (35.5%). Interestingly, of the TRV's, NC/SEP-R44/03 has proved to be the most pathogenic in turkeys. As a group, the TRVs share only about 53% to 56% amino acid sequence identity with S1133, which lends support to the previous observation that the TRVs may represent a separate virus species within the *Orthoreovirus* genus [4].

S1 genome segment gene arrangement

Although the TRV σC, p10, and p17 proteins appear to be quite divergent from other avian reoviruses and the fusogenic mammalian reoviruses, in the case of TRV

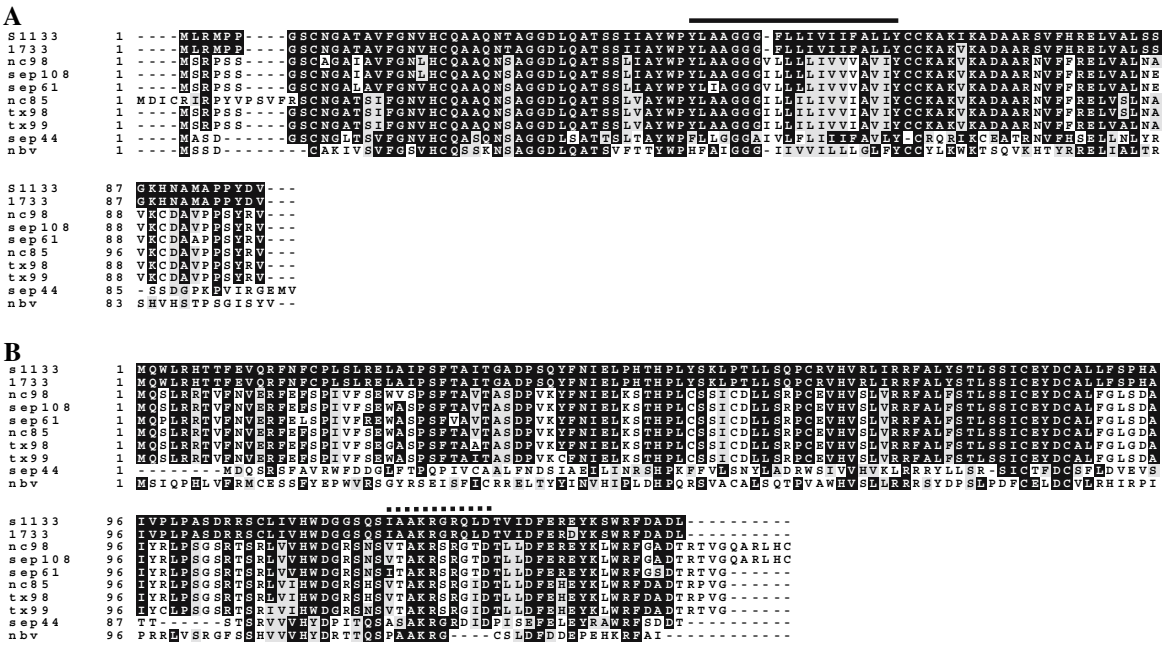

Fig. 4 Multiple sequence alignment of reovirus p10 (A.) and p17 (B.) proteins. The predicted p10 transmembrane motif is overscored with a solid black line, and the predicted p17 NLS is overscored by a dashed black line. Residues identical to the S1133 p10 or p17 amino acid sequence are shaded in black, and gray shading indicates conservative amino acid substitutions

isolates NC/SEP-R44/03 and NC/98 the basic gene arrangement of the TRV S1 genome segment remains the same. The three genes of the TRV S1 genome segments are partially overlapping and are arranged in the same order as the S1-encoded genes from other sequenced ARV isolates and NBV [1]. Further, the deduced σC proteins from the seven TRVs included in this study were similar in size to the cell attachment protein from other sequenced ARVs and NBV (about 326 residues), with the exception of the NC/SEP-R44/03 σC, which is 329 residues long.

The TRV cell attachment protein

While the NC/SEP-R44/03 σC protein stands out in this analysis as highly divergent from the other TRV cell attachment proteins, it is important to note that the NC/SEP-R44/03 σC retains an apolar residue at nearly every conserved position within the N-terminal heptad repeat motif. This coiled-coil heptad repeat motif is conserved in avian as well as mammalian reovirus cell attachment proteins and is important for the stability of the functional σC homotrimer and for its proper incorporation into virions [19–22]. Additionally, several aromatic residues conserved among the ARVs and NBV are either completely conserved in NC/SEP-R44/03 or are conservatively substituted (Fig. 3). These aromatic residues are found in the carboxy terminal globular ''head'' of the avian reovirus cell attachment protein. It is not presently known which residues in the avian reovirus carboxy terminus are important for receptor binding, but the recent avian reovirus σC crystal structure may help to identify regions important in host-virion interactions [7, 8].

The TRV σC proteins are very similar to one another (Fig. 2), and most group together in a phylogenetic analysis, but the NC/SEP-R44/03 σC is quite divergent even from the group of TRVs included in this study (Fig. 5). RT-PCR primers designed to detect the TRVs based upon sequence in the S3 genome segment do detect the NC/SEP-R44/03 strain, as well as the other six TRV strains included in this study, suggesting that the NC/SEP-R44/03 S3 genome segment, which encodes the major outer-capsid protein gene σB (sometimes reported as σ2), is quite similar to the S3 genome segment from the other TRVs [23].

Although much of the TRV genome remains to be sequenced and analyzed, based on the proposed function of the ARV σC gene and the pattern with which the TRV isolates infect chickens, the TRV σC protein may be involved with host specificity. Previous work with the TRVs indicates that certain isolates are not

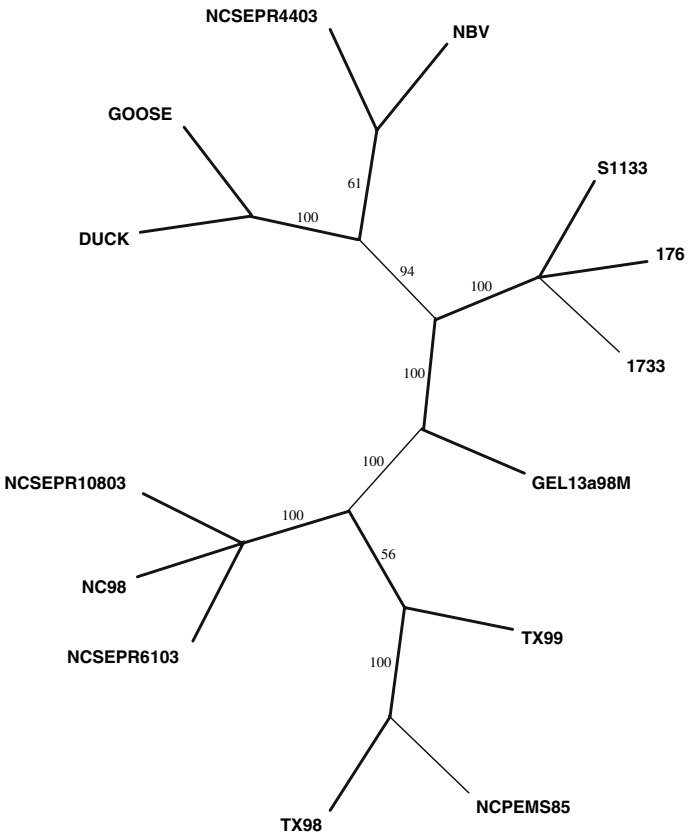

Fig. 5 Phylogenetic tree representing the relationships between the σC proteins from turkey-origin reoviruses and several other avian and mammalian fusogenic orthoreoviruses. This unrooted tree was generated using maximum parsimony and bootstrap confidence levels following 2000 replicates are indicated next to branches

likely to replicate in chickens as there was no measurable antibody response in experimentally exposed chickens, and infectious virus could not be recovered from these birds [6]. However, TRV isolates identified with S1133 σC-specific primers could replicate in chickens.

The TRV strains NC/PEMS/85 and TX/98 can be detected with primers designed to detect the S1 genome segment from the highly pathogenic chicken reovirus strain 1733 and S1133, but NC/PEMS/85 and TX/98, along with TRV strains NC/SEP-R108/03, NC/SEP-R44/03, NC/98, and TX/99, do not cause disease or cause the production of antibody in chickens [6]. The ARV strains 1733 and S1133 can replicate in turkeys without causing clinical disease, while in commercial turkey poults and specific pathogen free (SPF) turkey poults, TRV infection generally results in mild clinical signs. NC/SEP-R44/03 does cause severe bursal atrophy in SPF poults, and bursal lesions are associated with immunosuppression, a condition that could predispose poults to further infections leading to polymicrobial enteric syndromes such as poult enteritis complex (PEC) [6]. The role of the highly divergent NC/SEP-R44/03 σC protein in the progression, severity, or tissue specificity of TRV, however, remains to be determined. Tissue tropism and pathogenicity during mammalian reovirus infection appears to be dependent in part upon the σ1 cell attachment protein [24–26] and tissue tropism and invasiveness are known to affect the pathogenicity of ARVs [27]. Interestingly, a survey of the σC genes from Dutch and German chicken reovirus isolates showed no correlation between σC sequence and the disease state of the bird (i.e., malabsorption syndrome, tenosynovitis) from which isolates were obtained [11].

TRV p10 and p17 proteins

The divergence noted for the TRV cell attachment protein continues when the deduced amino acid sequence of the p10 and p17 genes is analyzed. Once again, this is particularly true for the NC/SEP-R44/03 sequence (Fig. 4). The p10 protein of the fusogenic reoviruses represents a completely new class of fusion-inducing proteins. All of the TRV p10 proteins included in this analysis contain a single residue insertion in their putative transmembrane domains (Fig. 4A). The TRV p17 proteins as a group are more divergent than the p10 proteins, which is in agreement with a survey of the p10 and p17 sequences of reoviruses isolated from chickens and quail, in which the p17 encoding gene was more divergent than the p10 encoding gene. Further, similar to the Dutch and German survey of avian reovirus σC sequences, the authors found no correlation between the p10 or p17 sequence and avian reovirus serotype or disease state [28].

The orthoreoviruses comprise a highly divergent genus that infects a wide range of animals [29]. This first look at the S1 genome segments and encoded proteins from a newly described group of reoviruses strongly suggests, along with previous work on the TRV σB (σ2) gene, that the turkey reoviruses are unique among the avian orthoreoviruses. This notion is further bolstered by pathogenesis studies that reveal that the TRVs at most can replicate at very low levels in chickens with no subsequent disease state. Cross reactivity of the TRVs with a commercial chicken reovirus antibody in an enzyme linked immunosorbent assay (ELISA) for antibody to S1133-like isolates suggests that these TRV isolates may have been identified as chicken isolates in the past [6]. Further molecular characterization of the remaining TRV genome segments is needed along with continued pathogenesis studies in order to determine the role the TRVs may play in poultry enteric disease and their prevalence in commercial flocks.

References

1. M. Shmulevitz, Z. Yameen, S. Dawe, J. Shou, D. O'Hara, I. Holmes, R. Duncan, J. Virol. **76**, 609 (2002)
2. J.K. Rosenberger, in *Diseases of Poultry* 11th edn., ed. by Y.M. Saif (Blackwell, Ames, IA, 2003), pp. 283–298
3. D.R. Kapczynski, H.S. Sellers, V. Simmons, S. Schultz-Cherry, Virus Genes **25**, 95 (2002)
4. H.S. Sellers, E.G. Linnemann, L. Pereira, D.R. Kapczynski, Avian Dis. **48**, 651 (2004)
5. H.J. Barnes, J.S. Guy, in Diseases of poultry, 11th edn., ed. by Y.M Saif, et al., (Blackwell, Ames, IA, 2003), pp. 1171–1180
6. E. Spackman, M.J. Pantin-Jackwood, J.M. Day, H.S. Sellers, Avian Path **34**, 291 (2005)
7. P. Guardado Calvo, G.C. Fox, X.L. Hermo Parrado, A.L. Llamas-Saiz, C. Costas, J. Martinez-Costas, J. Benavente, M.J. van Raaij, J. Mol. Biol. **354**, 137 (2005)
8. M.J. van Raaij, X.L. Hermo Parrado, P. Guardado Calvo, G.C. Fox, A.L. Llamas-Saiz, C. Costas, J. Martinez-Costas, J. Benavente, Acta Crystallog. Sect F **61**, 651 (2005)
9. M. Shmulevitz, R. Duncan, EMBO J. **19**, 902 (2000)
10. C. Costas, J. Martinez-Costas, G. Boledon, J. Benavente, J. Virol. **79**, 2141 (2005)
11. A. Kant, F. Balk, L. Born, D. Van Roozelaar, J. Heijmans, A. Gielkens, A. Ter Huurne, Vet. Res. **34**, 203 (2003)
12. J.K. Rosenberger, F.J. Sterner, S. Botts, K.P. Lee, A. Margolin, Avian Dis. **33**, 535 (1989)
13. R. Duncan, F.A. Murphy, R.R. Mirkovic, Virology **212**, 752 (1995)
14. R. Duncan, J. Corcoran, J. Shou, D. Stoltz, Virology **319**, 131 (2004)
15. G. Gard, R.W. Compans, J. Virol. **6**, 100 (1970)

16. Hung-Jen Liu, J.J. Giambrone, J. Virol. Methods **63**, 203 (1997)
17. M.L. Nibert, L.A. Schiff, in Fields Virology, 4th edn., ed. by D.M. Knipe, P.M. Howley, (Williams & Wilkins, Lippincott, 2001), pp. 1679–1728
18. Y. Vasserman, D. Eliahoo, E. Hemsani, N. Kass, G. Ayali, S. Pokamunski, J. Pitcovski, Avian Diseases **48**, 271 (2004)
19. R. Bassel-Duby, A. Jayasuriya, D. Chatterjee, N. Sonenberg, J.V. Maizel Jr., Nature **315**, 421 (1985)
20. R. Duncan, D. Horne, L.W. Cashdollar, W.K. Joklik, P.W.K. Lee, Virology **174**, 399 (1990)
21. G. Leone, D.C.W. Mah, P.W.K. Lee, Virology **182**, 346 (1991)
22. G. Leone, R. Duncan, D.C.W. Mah, A. Price, L.W. Cashdollar, P.W.K. Lee, Virology **182**, 336 (1990)
23. E. Spackman, D. Kapczynski, H. Sellers, Avian Diseases **49**, 86 (2005)
24. B.L. Haller, M.L. Barkon, G.P. Volger, H.W. Virgin, J. Virol. **69**, 357 (1995)
25. K.M. Kaye, D.R. Spriggs, R. Bassell-Duby, B.N. Fields, K.L. Tyler, J. Virol. **59**, 90 (1986)
26. D.R. Spriggs, R.T. Bronson, B.N. Fields, Science **220**, 505 (1983)
27. D.E. Roessler, J.K. Rosenberger, In vitro and in vivo characterization of avian reoviruses. III. Host factors affecting virulence and persistence. Avian Dis. **33**, 555 (1989)
28. H.W. Hsu, H.Y. Su, P.H. Huang, L.H. Lee, H.J. Liu, Avian Dis **49**, 36 (2005)
29. R. Duncan, Virology **260**, 316 (1999)
30. J.D. Thompson, D.G. Higgins,T.J. Gibson, Nucleic Acids Res. **22**, 4673 (1994)
31. J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, Nucleic Acids Res. **24**, 4876 (1997)
32. D.L. Swofford, PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4 (Sinauer Associates, Sunderland, MA, 2002)